

(2)

EUROPEAN PATENT APPLICATION

(2) Application number: 90302932.0

(2) Int. Cl.5 G01N 27/416, C12M 1/40

(2) Date of filing: 19.03.90

(2) Priority: 20.03.89 US 325389
11.01.90 US 463540

(2) Date of publication of application:
03.10.90 Bulletin 90/40

(2) Designated Contracting States:
CH DE FR GB IT LI NL SE

(7) Applicant: ASSOCIATED UNIVERSITIES, INC.
Upton
Long Island, New York 11973(US)

Applicant: POLYTECHNIC UNIVERSITY
333 Jay Street
Brooklyn New York 11201(US)

(7) Inventor: Skothelm, Terje A.
PO Box 675, Woodville Road
Shoreham, New York 11786(US)
Inventor: Hala, Paul D.
5 Kow Avenue
Northport, New York 11731(US)
Inventor: Okamoto, Yoshiyuki
3 Horizon Road
Fort Lee, New Jersey 07024(US)

(7) Representative: Bankes, Stephen Charles
Digby
BARON & WARREN 18 South End Kensington
London W8 5BU(GB)

(2) Electrochemical biosensor based on immobilized enzymes and redox polymers.

(2) The present invention relates to an electrochemical enzyme biosensor for use in liquid mixtures of components for detecting the presence of, or measuring the amount of, one or more select components. The enzyme electrode of the present invention is comprised of an enzyme, an artificial redox compound covalently bound to a flexible polymer backbone and an electron collector.

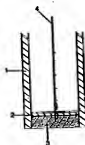


Fig. 1

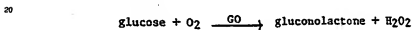
ELECTROCHEMICAL BID SENSOR BASED ON IMMOBILIZED ENZYMES AND REDOX POLYMERS

The present invention relates to an enzyme electrode or electrochemical biosensor, which may be used for electrochemically measuring the concentration of and/or monitoring the level of one or more selected components in a liquid mixture or liquid environment.

- These electrochemical biosensors may be used in the chemical, food and biochemical industries and in clinical applications in animal or human medicine, in particular to measure in vivo the concentration of components in body fluids.

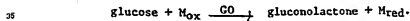
These electrochemical biosensors will be described with reference to one particular measurement, the determination of the glucose concentration in an aqueous mixture. While the measurement of glucose concentration is one object of the invention, other and broader objects are not hereby excluded.

- Typical amperometric glucose electrodes based on glucose oxidase (GO) undergo several chemical or electrochemical steps which produce a measurable current which is linearly related to the glucose concentration. In the initial step, glucose converts the oxidized flavin adenine dinucleotide (FAD) center of the enzyme into its reduced form (FADH₂). Because these redox centers are located well within the enzyme molecule, direct electron transfer to the surface of a conventional electrode does not occur to any measurable degree. A common method of indirectly measuring the amount of reduced glucose oxidase, and hence the amount of glucose present, relies on the natural enzymatic reaction as described in Biosensors: Fundamentals and Applications (Oxford University Press, New York, 19873 chapter 1, and shown by the following reaction formula:



- In this reaction, oxygen is the electron acceptor for glucose oxidase. Glucose is oxidized (dehydrogenated) to gluconolactone through the catalytic reaction caused by glucose oxidase, while oxygen is reduced to H₂O₂. The concentration of O₂ and H₂O₂ can be measured with conventional electrochemical techniques, whereby it is possible to obtain the concentration of glucose indirectly by means of the measurement of O₂ consumption as well as H₂O₂ formation governed by the reaction depicted above. In this measuring scheme the sensor has the disadvantage of being sensitive to the concentration of O₂.

- Instead of the natural electron acceptor O₂, an artificial electron acceptor (mediator) may be used to shuttle electrons between the reduced flavin adenine dinucleotide and the electrode by the following mechanism, described in Anal. Chem. 56, 667-671 (1984):



The preferred mediating species M may be, but is not limited to, ferrocene or a substituted ferrocene.

- U.S. Patents 4,545,382 and 4,711,245 disclose an enzyme electrode, in which the mediator is a ferrocene or substituted ferrocene molecule. In potential clinical applications, or where long term stability is desirable, sensors based on electron-shuttling redox couples suffer from an inherent drawback: the soluble, or partially soluble, mediating species can diffuse away from the electrode surface into the bulk of the solution. This precludes the use of these devices in implantable probes.

- U.S. Patent No. 4,224,125 discloses an enzyme electrode, in which the water soluble mediator is in polymeric form in order to remain immobilized near the electrode surface by being too large to diffuse through a retaining membrane into the bulk of the solution. The polymeric redox mediator is reduced by the enzyme catalytic process and reoxidized by the electrode, in the vicinity of which it is contained. This electrode design requires a retaining membrane which is a disadvantage for microelectrode applications or where rapid response and high sensitivity are important features of the electrode.

- It is desirable to find a mediator which can rapidly transfer electrons between the enzyme and the electrode at a rate corresponding to the rate of the enzyme-catalyzed reaction.

It is further desirable to use a mediating species which is covalently attached in such a fashion as to make it insoluble in the solution to be analyzed, thus preventing the mediating species from diffusing away from the electrode surface.

It is specifically desirable to find a mediator which is relatively insensitive to the presence of interfering

substances, in particular oxygen.

These objects are accomplished by the present invention wherein the mediating species is chemically bound to a flexible polymer backbone which allows close contact between the FAD/FADH₂ centers of the enzyme and the attached mediator, yet prevents the latter from diffusing away from the electrode surface.

- 5 The present invention covers a class of redox polymers which has exceptional properties for mediating enzyme-catalyzed reactions in electrode sensing systems. The redox polymer acts as an electron transfer relay system in a manner similar to that described by Degani and Heller [*J. Phys. Chem.*, **91**, 1285-1289 (1987)] where the electron relays are covalently attached to the enzyme itself. A disadvantage of this prior art design is measurably reduced enzyme activity. A further disadvantage is that the applicability of this design is limited to enzymes which allow this particular attachment chemistry. In the present invention, the necessary electrical communication between the FAD/FADH₂ centers and the electrode is achieved without modifying the enzyme. A key aspect of the present invention is the use of a highly flexible polymer backbone with sufficient local conformational mobility to allow the attached mediator species to come in close proximity to the enzyme catalytic center, thereby acting as an efficient electron transfer relay to an
- 10 electron collector. The present system is applicable to all oxido-reductase enzymes, including enzymes which have been modified according to the scheme of Degani and Keller.

- One aspect of the invention is to provide a network of donor/acceptor relays covalently attached to a flexible polymer backbone. In another aspect of the invention the flexible polymer backbone is provided by a siloxane polymer. The unique flexibility of the polysiloxane backbone, which has virtually no energy barrier to rotation, allows these relay moieties to interact intimately with the enzyme molecule and achieve a close contact with the FAD/FADH₂ centers. This is a vital consideration since, as has been demonstrated [*J. Electroanal. Chem.*, **250**, 417-425 (1988)], less flexible redox polymers such as poly(vinylferrocene) cannot achieve a sufficiently close contact with the enzyme's redox centers to serve as effective electron transfer relay systems.

- 25 An object of the invention is to provide an enzyme electrode for use in liquid mixtures of components for detecting the presence of, measuring the amount of and/or monitoring the level of one or more selected components capable of undergoing an enzyme-catalyzed reaction, in which an oxido-reductase enzyme as well as a polymeric mediator system which transfers electrons to an electron collector when the enzyme is catalytically active, are both maintained in an immobilized state on at least an external surface of the electron collector.

A further object of the invention is to provide an enzyme electrode of the above described type in which the polymeric mediator is comprised of a flexible polymer backbone onto which is covalently attached molecular mediator compounds such as to form a donor-acceptor electron relay system.

- A further object of the invention is to provide a polymeric mediator compound which is insoluble in aqueous mixtures such as to remain immobilized on the electrode surface without a retaining membrane.

A further object of the present invention is to provide an enzyme electrode of the above described type which can be formed on a small scale to be used for in vivo concentration measurements.

The invention will be further described with reference to the accompanying drawings, in which:

- Fig. 1 is a schematic diagram partially showing a longitudinal cross-section of an enzyme electrode according to the present invention.

Fig. 2 is a graph showing the current sensed by the electrode of Fig. 1, against glucose concentration.

The electrochemical biosensor of the present invention is characterized by high efficiency for transferring electrons from the reduced enzyme to an electron collector using an artificial redox compound, which functions as a non-physiological electron transfer mediator. The high efficiency derives from the highly flexible polymeric backbone which allows local conformational mobility, thereby providing close proximity to the enzyme redox center of the mediator compounds which, are covalently attached to the polymer backbone. The enzyme redox center is located too deep within the enzyme to perform direct electron transfer to a conventional electrode surface.

- 30 The preferred polymer backbone is a siloxane polymer, which is characterized by having virtually no energy barrier to rotation, thereby allowing facile local segmental motion. Other examples of flexible polymer backbones are polyphosphazene, poly(ethylene oxide) and poly(propylene oxide).

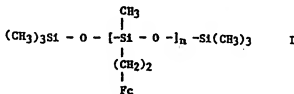
The preferred mediator compounds are metallocenes, which are organometallic compounds comprising two organic ring structures, each with conjugated unsaturation, and a metal atom sandwiched between the rings, so that the metal atom is in electron-sharing contact with the unsaturated rings.

- 35 Ferrocene (dicyclopentadienyl iron), and substituted ferrocene compounds are particularly effective mediators, having pH-independent electrochemically reversible one-electron redox properties, a pH-independent redox potential, slow autooxidation of the reduced form, the absence of any known problems of

toxicity or carcinogenicity, a redox potential sufficiently low to avoid excessive interference from competing higher redox potential reactions, satisfactory oxygen insensitivity to avoid excessive interference from oxygen and the ability to be covalently attached to polymer backbones. In a preferred embodiment, no low molecular weight ferrocene species are present in the polymer since such species could act as freely diffusing electron transfer mediators.

A further advantage of the ferrocene mediating compounds is the ability to control the redox potential over a wide range through substitution of electron donating or withdrawing groups on the cyclopentadienyl rings. Preferred substituted ferrocenes include, but are not limited to, 1,1-dimethylferrocene, vinylferrocene, hydroxyethylferrocene, 1,1'-bis(hydroxymethyl)ferrocene, carboxyferrocene, ferrocenylmonocarboxylic acid, 1,1'-dicarboxyferrocene, and trimethylaminoferrocene.

A preferred polymeric mediator system based on a siloxane backbone and attached ferrocene or substituted ferrocene mediator compound is exemplified by the following structural formula:



wherein Fc is ferrocenyl or substituted ferrocenyl, and $n = 10$ to 50 , with $n \approx 35$ being preferred.

Other preferred mediator compounds include ruthenocene, dibenzene chromium, phenazine and phenazine derivatives, viologen, riboflavin, p-benzoquinone, and naphthoquinone. In general, redox compounds which can be covalently attached to polymeric backbones and which have redox potentials in the range -0.2 to 0.8 V vs. the Saturated Calomel electrode (SCE) are applicable.

The ferrocene and substituted ferrocene compounds are particularly applicable, as the ferrocenes can mediate electron transfer for a broad range of enzymes.

The preferred enzymes are non-oxygen-specific flavo-protein or quino-protein enzymes, in particular glucose oxidase and glucose dehydrogenase. Other flavo-protein enzymes include aldehyde oxidase (aldehydes), glycolate oxidase (glycolate), glutathione reductase (NAD(P)H), lactate oxidase (lactate), L-amino acid oxidase (L-amino acids), lipamide dehydrogenase (NADH), pyruvate oxidase (pyruvate), sarcosine oxidase (sarcosine), choline oxidase (choline) and xanthine oxidase (xanthine), where the substrate to which the enzyme is specific has been denoted in parenthesis.

Other quino-protein enzymes include methylamine dehydrogenase (methylamine) and methanol dehydrogenase (methanol and other alcohols).

Heme-containing enzymes which can be oxidized by ferrocenes include horse-radish peroxidase (hydrogen peroxide), lactate dehydrogenase (lactate) and yeast cytochrome C peroxidase (hydrogen peroxide).

The cupro-protein enzyme galactose oxidase (galactose) and the metalloflavin protein enzyme carbon monoxide oxidoreductase (carbon monoxide) are also applicable.

The enzyme electrodes may be constructed by mixing graphite powder, siloxane-ferrocene polymer and glucose oxidase and blending the resultant mixture into a paste which is subsequently packed into a well at the base of an electrode housing, as shown schematically in Fig. 1.

In order to achieve long term stability, it is advantageous to covalently immobilize the enzyme to the siloxane polymer backbone. This can be achieved with the method described in Biosensors 3, 45-56 (1987/88), with amine groups selectively attached to the siloxane backbone on some of the polymer chains or alternately with ferrocenes on the same polymer chain.

The preferred electron collector material is graphite paste due to ease of fabrication and large surface area. Other electrode materials may be silver, platinum, nickel, glassy carbon or tin-oxide.

The manner in which the enzyme electrodes of the present invention are constructed can be understood more fully by reference to the following illustrative examples.

EXAMPLE 1

Glucose oxidase/siloxane-ferrocene polymer

In the following embodiment of the present invention the enzyme was glucose oxidase and the polymer is a siloxane-ferrocene polymer of formula I above wherein Fc is ferrocenyl and n is approximately 35.

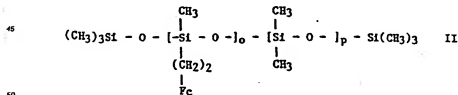
This ferrocene-modified siloxane homopolymer was prepared by the hydrosilylation of vinylferrocene with poly(methylhydrosiloxane). Under nitrogen atmosphere, poly(methylhydrosiloxane) (molecular weight 2270) was added into toluene solution of an excess amount of vinylferrocene in the presence of chloroplatinic acid. The reaction mixture was then heated to the reflux temperature. The reaction was allowed to continue until the Si-H IR absorption band disappeared, indicating that all of the starting polymer was converted to product. The resulting ferrocene-modified siloxane polymer was purified by reprecipitation from a chloroform solution via dropwise addition into a large excess of acetonitrile at room temperature. This reprecipitation was repeated until thin layer chromatography showed that no vinylferrocene was present in the precipitate.

Referring to the drawings, Fig. 1 shows the structural details of the enzyme electrode of the present invention. The enzyme electrode comprises a cylindrical electrode holder 1 of an electrically insulating material, an electron collector 2 of carbon formed in a disc-like configuration and mounted recessed in the electrode holder 1, a leading wire 4 connected to the electron collector 2, and a carbon paste 3 containing the enzyme-polymer system. The carbon paste was constructed by thoroughly mixing 100 mg of graphite powder with 1 mg of the ferrocene containing polymer, the latter being dissolved in chloroform. After evaporation of the solvent, 10 mg of glucose oxidase (129,000 units/mg) and 20 μ l of paraffin oil were added, and the resulting mixture blended into a paste. The paste was packed into a 2 mm deep recess at the base of a glass electrode holder (6 mm inner diameter). For the measurement of the current response the reference electrode was a saturated calomel electrode (SCE) and the auxiliary electrode consisted of a platinum wire. The solutions were deoxygenated with nitrogen prior to each experiment.

In Fig. 2, there is shown a current response curve giving the variation of the current measured as a function of the concentration of glucose in a pH 7.0 phosphate buffer solution with 0.1 M KCl added. The enzyme electrode was connected to a potentiostat and maintained at a constant potential of 400 mV vs. the SCE reference electrode. The current produced is proportional to the glucose concentration. The time for 95% of response is approximately 2 minutes.

EXAMPLE 2Glucose oxidase/siloxane-ferrocene copolymer

In the following embodiment the enzyme was glucose oxidase and the polymer a copolymer having the following structural formula:



wherein Fc is as defined above, the op ratio is approximately 1:2 and o + p is equal to or greater than 10, with the subunits being randomly distributed to form a random block copolymer.

The co-polymer of this embodiment has a lower ferrocene density than the homopolymers of formula 1. The decreased steric hindrance results in a more flexible polymer with more facile local segmental motion to better provide close contact between the ferrocenes and the enzyme redox centers. The experimental results, using glucose oxidase/methyl(ferrocenyl ethyl)dimethyl (1:2) siloxane copolymer showed that the increase in current for a specific glucose concentration of 10^{-2} mol/l was 35 μ A, which is an improvement

over the response in Example 1 by approximately a factor of 3.

EXAMPLE 3

Glycolate oxidase/siloxane-ferrocene polymer

10 A platinum wire (diameter: 0.01 inch) was dipcoated with the ferrocene-modified siloxane polymer of Example 1 from a solution of 10 mg/ml, then with glycolate oxidase (30 units/ml in pH 8.3 Tris buffer). The current response to sodium glycolate was measured at a potential of 500 mV vs SCE in a solution comprising a phosphate buffer, 0.1 M KCl at pH 8.7. The solution was deaerated with N₂. Adding 30 mM
15 sodium glycolate resulted in a current of 0.11 μ A.

While particular embodiments of the invention have been described, it will be understood, of course, that the invention is not limited thereto, and that many obvious modifications and variations thereof can be made by those skilled in the art, and that such modifications and variations are intended to fall within the scope of the appended claims.

20

Claims

1. An enzyme electrode for sensing the presence of at least one component of a mixture of
25 components, said enzyme electrode comprising:
 - (a) an enzyme, the catalytic activity of said enzyme being indicative of said component,
 - (b) an artificial redox compound acting as an electron transfer mediator between the enzyme and electron collector, said mediator being covalently attached to an insoluble, flexible polymer backbone in sufficient number to form an electron relay system,
 - 30 (c) and an electron collector, said enzyme and said mediator-containing polymer being in an immobilized state in contact with said electron collector.
2. The enzyme electrode of claim 1, wherein the polymer backbone is a siloxane.
3. The enzyme electrode of claim 1, wherein the redox compound is ferrocene or a substituted
ferrocene.
- 35 4. The enzyme electrode of claim 3, wherein the redox compound is selected from the group consisting of ferrocene, 1,1'-dicarboxyferrocene, carboxyferrocene, vinylferrocene, 1,1'-dimethylferrocene, ferrocenyl-monocarboxylic acid, hydroxyethylferrocene, and 1,1'-bis(hydroxymethyl)ferrocene.
5. The enzyme electrode of claim 1, wherein the redox compound is ferrocene or a substituted ferrocene, the polymer backbone is a siloxane, and the redox compound is covalently attached to the
40 polymer backbone.
6. The enzyme electrode of claim 1, wherein the enzyme is glucose oxidase.
7. The enzyme electrode of claim 1, wherein the enzyme is glycolate oxidase.
8. The enzyme electrode of claim 6 for use in a liquid mixture, including glucose, to be responsive to the presence of glucose.
- 45 9. The enzyme electrode of claim 7 for use in a liquid mixture, including glycolate, to be responsive to the presence of glycolate.
10. The enzyme electrode of claim 8, wherein the polymer backbone is a siloxane and the enzyme is covalently attached to the polymer backbone.
11. The enzyme electrode of claim 1, wherein the polymer backbone is siloxane, the redox compound
50 is ferrocene, and the enzyme is glucose oxidase.
12. The enzyme electrode of claim 11, wherein the electron collector is composed of graphite powder, and said glucose oxidase, said polymer and said ferrocene are located on the external surface of said graphite powder.
13. The enzyme electrode of claim 11 having a protective membrane permeable to water and glucose
55 molecules, said membrane covering said external surface of said graphite electron collector.
14. The enzyme electrode of claim 6, wherein the electron collector is composed of platinum or solid carbon.
15. The enzyme electrode of claim 1 comprising means for implantation of said enzyme electrode in a

EP 0 390 390 A1

human subject.

5

10

15

20

25

30

35

40

45

50

55

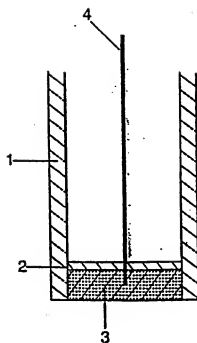


Fig.1

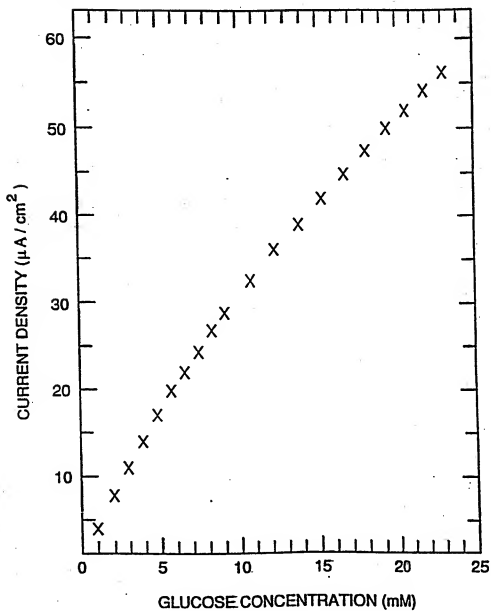


Fig. 2



European Patent
Office

EUROPEAN SEARCH REPORT

Application Number

EP 90 30 2932

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
P, X	JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol. 111, no. 9, 1989, pages 3482-3484, American Chemical Society, Columbus, Ohio, US; P.D. HALE et al.: "A new class of amperometric biosensor incorporating a polymeric electron-transfer mediator" * The whole document *	1-15	G 01 N 27/416 C 12 M 1/40
X	ANALYTICAL CHEMISTRY, vol. 60, 1988, pages 2473-2478, American Chemical Society, Columbus, Ohio, US; N.C. FOULDS et al.: "Immobilization of glucose oxidase in ferrocene-modified pyrrole polymers" * Experimental section; conclusions *	1,3,4,6 8,14	
A	JOURNAL OF THE CHEMICAL SOCIETY, CHEMICAL COMMUNICATIONS, no. 15, 1988, pages 1019-1020, London, GB; C. IWAKURA et al.: "Simultaneous immobilization of glucose oxidase and a mediator in conducting polymer films" * The whole document *	1-14	TECHNICAL FIELDS SEARCHED (Int. Cl.5) G 01 N C 12 M
A	CHEMICAL ABSTRACTS, vol. 110, 1989, page 348, abstract no. 110990v, Columbus, Ohio, US; F. MIZUTANI et al.: "Ferrocene-mediated enzyme electrode for glucose with the use of conducting polymer support", & BULL. CHEM. SOC. JPN. 1988, 61(12), 4458-60 * The whole abstract *	1-14	
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 28-06-1990	Examiner EPAILLARD P.J.H.M.
CATEGORY OF CITED DOCUMENTS		T: theory or principle underlying the invention E: earlier patent document, but published on, or after the filing date D: document cited in the application L: document cited for other reasons A: members of the same patent family, corresponding document X: particularly relevant if taken alone Y: particularly relevant if combined with another document of the same category A: technological background O: oral-written disclosure P: intermediate document	

EP 90 30 2932 (P.04)

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☒ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.



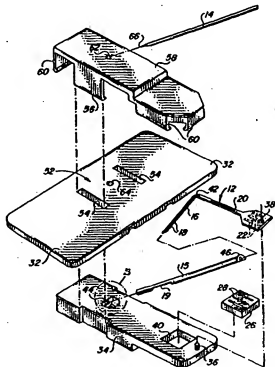
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61B 5/00	(11) International Publication Number: WO 96/25089 (43) International Publication Date: 22 August 1996 (22.08.96)
A1	
(21) International Application Number: PCT/US96/02006	(81) Designated States: CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).
(22) International Filing Date: 14 February 1996 (14.02.96)	Published With international search report.
(30) Priority Data: 08/393,159 16 February 1995 (16.02.95) US	
(71) Applicant: MINIMED INC. [US/US]; 12744 San Fernando Road, Sylmar, CA 91342 (US).	
(72) Inventors: CHENEY, Paul, S., II; 320 N. Palm Drive #215, Beverly Hills, CA 90210 (US); MASTROTOTARO, John, J.; 1235 Brockton Avenue #106, Los Angeles, CA 90025 (US); SCHNABEL, Nannette, M.; 24101 W. Del Monte Drive #418, Valencia, CA 91355 (US); LORD, Peter, C.; 25505 Ol Course Way, Valencia, CA 91355 (US); VAN ANTWERP, William, P.; 24101 W. Del Monte Drive #418, Valencia, CA 91355 (US); CLARK, Raymond, D.; 23734 Via Tomas, Valencia, CA 91355 (US).	
(74) Agent: LOWRY, Stuart, O.; Kelly, Bauersfeld & Lowry, Suite 1650, 6320 Canoga Avenue, Woodland Hills, CA 91367 (US).	

(54) Title: TRANSCUTANEOUS SENSOR INSERTION SET

(57) Abstract

An insertion set (10) is provided for transcutaneous placement of a sensor (12) such as a glucose sensor at a selected site within the body of a patient. The insertion set (10) comprises an insertion needle (14) extending through a mounting base (34) adapted for mounting onto a patient's skin. A flexible thin film sensor (12) includes a proximal segment (20) carried by the mounting base (34) and adapted for electrical connection to a suitable monitor and a distal segment (16) protruding from the mounting base (34) with sensor electrodes (18) thereon for transcutaneous placement. The distal segment (16) of the sensor (12) and a distal segment (66) of the insertion needle (14) are positioned within a flexible cannula (15) which extends from the mounting base (34), whereby placement of the mounting base (34) onto the patient's skin causes insertion needle (14) to pierce the skin for transcutaneous placement of the cannula (15) with the sensor (12) therein.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Ghana	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LJ	Liechtenstein	SI	Slovenia
CI	Cote d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

TRANSCUTANEOUS SENSOR INSERTION SETBACKGROUND OF THE INVENTION

This invention relates generally to devices and methods for placing a sensor at a selected site within the body of a patient. More specifically, this invention relates to an improved and relatively simple insertion set for quick and easy transcutaneous placement of a flexible thin film sensor of the type used, for example, to obtain periodic blood glucose readings.

In recent years, a variety of electrochemical sensors have been developed for a range of applications, including medical applications for detecting and/or quantifying specific agents in a patient's blood. As one example, glucose sensors have been developed for use in obtaining an indication of blood glucose levels in a diabetic patient. Such readings can be especially useful in monitoring and/or adjusting a treatment regimen which typically includes regular administration of insulin to the patient. In this regard, blood glucose readings are particularly useful in conjunction with semiautomated medication infusion pumps of the external type, as generally described in U.S. Patents 4,562,751; 4,678,408; and 4,685,903; or automated implantable medication infusion pumps, as generally described in U.S. Patent 4,573,994.

Relatively small and flexible electrochemical sensors have been developed for subcutaneous placement of sensor electrodes in direct

-2-

contact with patient blood or other extracellular fluid, wherein such sensors can be used to obtain periodic readings over an extended period of time. In one form, flexible transcutaneous sensors are constructed in accordance with thin film mask techniques wherein an elongated sensor includes thin film conductive elements encased between flexible insulative layers of polyimide sheet or similar material. Such thin film sensors typically include exposed electrodes at a distal end for transcutaneous placement in direct contact with patient blood or the like, and exposed conductive contacts at an externally located proximal end for convenient electrical connection with a suitable monitor device. Such thin film sensors hold significant promise in patient monitoring applications, but unfortunately have been difficult to place transcutaneously with the sensor electrodes in direct contact with patient blood or other extracellular fluid. Improved thin film sensors and related insertion sets are described in commonly assigned copending U.S. Serial Nos. 08/213,101, filed March 14, 1994; 08/212,961, filed March 14, 1994; and 08/239,960, filed May 9, 1994, which are incorporated by reference herein. See also U.S. Patent 5,299,571.

The present invention relates specifically to an improved sensor insertion set adapted for quickly and easily placing a thin film sensor on a patient with sensor electrodes in direct contact with patient blood or other extracellular fluid.

SUMMARY OF THE INVENTION

In accordance with the invention, a subcutaneous insertion set is provided for placing a flexible sensor such as a thin film electrochemical sensor at a selected site within the body of a

patient. The insertion set comprises an insertion needle extending through a mounting base adapted for seated mounting onto the patient's skin. A flexible thin film sensor includes a proximal segment carried by the mounting base, and a distal segment protruding from the mounting base and having sensor electrodes thereon. The distal segment of the sensor and a distal segment of the insertion needle are carried within a hollow flexible cannula extending from the mounting base. When the mounting base is pressed onto the patient's skin, the insertion needle pierces the skin for transcutaneous placement of the cannula with the sensor therein. The insertion needle can then be withdrawn from the mounting base, leaving the sensor distal segment with the electrodes thereon exposed through a window or windows in the cannula for direct contact with patient fluid at the selected position within the patient, such as a subcutaneous, intravascular, intramuscular, or intravenous site. Conductive contacts on the sensor proximal end can be electrically connected to a suitable monitor device so that appropriate blood chemistry readings can be taken and monitored.

In one aspect of the invention, the cannula includes structural means which cooperates with the mounting base and/or the sensor distal segment to insure alignment of the sensor electrodes with the cannula window. More particularly, in the preferred form, a mounting flange at an upper end thereof, having a noncircular cross sectional shape such as a D-shaped cross section, for seated mounting within a matingly shaped recess formed in the mounting base. With this construction, the cannula is rotationally oriented relative to the mounting base in a predetermined manner, such that the window or windows therein are also oriented in a predetermined manner for proper alignment with the sensor electrodes. The distal segment of the sensor comprises a thin film

strip fitted within the cannula to lie against or adjacent an interior wall of the cannula with the sensor electrodes exposed preferably in a generally downward direction, through the cannula window.

The insertion needle, when assembled with the mounting base to extend through the hollow cannula, supports the sensor distal segment in a position pressed against the cannula wall during transcutaneous sensor placement. After withdrawal of the insertion needle, the cannula protects the sensor to maintain the position thereof within the patient. In addition, in one embodiment, the cannula provides a transcutaneous path for delivery of fluid to or withdrawal of fluid from the patient.

Other features and advantages of the present invention will become more apparent from the following detailed description, taken in conjunction with the accompanying drawings which illustrate, by way of example, the principles of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings illustrate the invention. In such drawings:

FIGURE 1 is a perspective view illustrating an upper side of a transcutaneous sensor insertion set embodying the novel features of the invention;

FIGURE 2 is an exploded perspective view showing the transcutaneous sensor insertion set of FIG. 1;

FIGURE 3 is an enlarged fragmented longitudinal section taken generally on the line 3-3 of FIG. 1;

FIGURE 4 is an enlarged fragmented transverse section taken generally on the line 4-4 of FIG. 1;

-5-

FIGURE 5 is an enlarged fragmented perspective view showing a portion of a mounting base for the sensor insertion set;

FIGURE 6 is an enlarged fragmented sectional view corresponding generally with the encircled region 6 of FIG. 3;

FIGURE 7 is a fragmented prospective view showing one alternative preferred form of the invention;

FIGURE 8 is a transverse section taken generally on the line 8-8 of FIG. 7;

FIGURE 9 is a fragmented perspective view showing another alternative preferred form of the invention;

FIGURE 10 is a transverse section taken generally on the line 10-10 of FIG. 9;

FIGURE 11 is a fragmented perspective view of a portion of a thin film sensor shown in FIGS. 7 and 8; and

FIGURE 12 is a fragmented perspective view of a portion of an alternative style thin film sensor shown in FIGS. 9 and 10.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

As shown in the exemplary drawings, an improved sensor insertion set referred to generally in FIGURE 1 by the reference numeral 10 is provided for transcutaneous placement of a flexible sensor 12 (FIG. 2) at a selected site within the body of a patient. The insertion set 10 includes a rigid insertion needle 14 for quick and easy transcutaneous placement of a flexible hollow cannula 15 with a distal segment 16 of the sensor 12 therein, wherein the distal segment 16 has one or more sensor electrodes 18 exposed to patient fluid through a window 19 in the cannula 15. The insertion needle 14

is then withdrawable to leave the sensor distal segment 16 with electrodes 18 thereon in place within the cannula 15, at the selected insertion site.

The transcutaneous sensor insertion set 10 of the present invention is particularly designed for facilitating accurate placement of a flexible thin film electrochemical sensor of the type used for monitoring specific blood parameters representative of patient condition. The insertion set 10 is designed to place the sensor subcutaneously or at another selected site within the body of a patient, in a manner minimizing patient discomfort and trauma. In one preferred application, the sensor 12 may be designed to monitor blood glucose levels, and may be used in conjunction with automated or semiautomated medication infusion pumps of the external or implantable type as described in U.S. Patents 4,562,751; 4,678,408; 4,685,903 or 4,573,994, to deliver insulin to a diabetic patient.

As shown best in FIGS. 2 and 3, the flexible electrochemical sensor 12 is a thin film sensor which may be constructed according to so-called thin film mask techniques to include elongated thin film conductors embedded or encased between upper and lower layers and of a selected insulative material such as polyimide film or sheet. The sensor electrodes 18 at a tip end of the distal segment 16 are exposed through one of the insulative layers for direct contact with patient blood, when the sensor is transcutaneously placed. The distal segment 16 is joined to a proximal segment 20, the end of which terminates in conductive contact pads 22 which are also exposed through one of the insulative layers. As is known in the art, and illustrated schematically in FIG. 1, these conductive contact pads 22 are adapted for electrical connection to a suitable monitor 24 for monitoring patient condition in

-7-

response to signals derived from the sensor electrodes. Further description of flexible thin film sensors of this general type may be found in copending U.S. Serial No. 08/212,961, filed March 14, 1994, entitled METHOD OF FABRICATING THIN FILM SENSORS, and U.S. Serial No. 08/239,960, filed May 9, 1994, entitled FLEX CIRCUIT CONNECTOR, which are incorporated by reference herein. In this regard, FIGS. 1-3 show connection of the sensor proximal segment 20 connected electrically to the monitor 24 by means of a connector block 26 having conductive strip elements 28 (FIG. 2) as described in U.S. Serial No. 08/239,960.

In general, the sensor 12 is carried by a mounting base 30 adapted for placement onto the skin of a patient. As shown, the mounting base 30 comprises an enlarged and generally rectangular mounting pad structure defining oppositely projecting wings 32 each having an underside surface coated with a suitable pressure sensitive adhesive. A peel-off paper strip (not shown) is normally provided to cover and protect the adhesive layer, until the insertion set 10 is ready for use.

More particularly, as shown in one preferred construction in FIGS. 1-4, the mounting base 30 comprises a central housing member 34 formed from a suitable medical grade and relatively stiff or rigid plastic material such as polycarbonate or the like. This central housing member 34 is adapted to receive and support the proximal segment 20 of the sensor 12 in a position with the contact pads 22 thereon for connection to the monitor 24 by means of the connector block 26 as previously described. A locator pin 36 (FIG. 2) may be provided on the housing member 34 for reception through a port 38 formed in the sensor proximal segment 20 to insure proper mounting position of the sensor. In addition,

FIG. 2 shows a square aperture 40 in the housing member 34 for seated reception of the connector block 26. The sensor proximal segment 20 is joined at a bend 42 to the distal segment 16 which protrudes downwardly through an open port 44 to terminate at a selected depth or spacing below the housing member 34.

As shown best in FIGS. 2-5, the flexible cannula 15 has a mounting flange 46 at an upper or proximal end thereof nested within a recess 48 in the housing member 34 at the port 44. The cannula mounting flange 46 has a noncircular cross sectional shape, such as a D-shaped configuration as shown, for mating unidirectional seated fit into the housing member recess 48. Accordingly, the cannula 15 is supported by the housing member 34 in a predetermined rotational orientation, to correspondingly orient the window 19 therein in a predetermined position relative to the mounting base. From the mounting flange 46, the cannula 15 protrudes through the port 44 in an angularly downward and forward direction with the distal segment 16 of the sensor 12 nested therein.

The attachment wings 32 of the mounting base 30 are formed by the opposite ends of a resilient wing member 52 (FIG. 2) having a size and shape to transversely overlie the central housing member 34. A central region of this wing member 52 has a pair of slots 54 formed thereon for pass-through reception of a downwardly projecting pair of snap-fit tabs 56 of an overlying cover plate 58. This cover plate 58 is formed from a suitable stiff or rigid material such as polycarbonate plastic, and includes additional snap-fit feet 60 for engaging and retaining the wing member 52 tightly against the underlying housing member 34, and thereby engage and retain the sensor proximal segment 20 tightly against the connector block 26.

The insertion needle 14 is adapted for slide-fit reception through circular needle ports 62 and 64 formed respectively in the cover plate 58, and through the wing member 52. As shown in FIGS. 1 and 2, the insertion needle 14 extends further through the cannula 15 alongside the sensor distal segment 16, to terminate in a sharpened tip 66 which protrudes a short distance beyond the cannula.

In accordance with a primary aspect of the invention, as shown in FIGS. 1-3, the mounting of the cannula 15 in the predetermined orientation relative to the housing member 34 results in the cannula windows or window 19 also being oriented in a predetermined manner, such as being exposed downwardly as viewed in the illustrative drawings. With this construction, the sensor distal segment 16 can be manufactured with the electrodes 18 thereon oriented for downward exposure, when the sensor is installed onto the mounting base 30 as shown. Accordingly, reliable and positive alignment of the sensor electrodes 18 with the cannula window 19 results to insure that the sensor electrodes 18 are properly exposed for direct contact with patient body fluid during use.

The insertion set 10 is installed quickly and easily by pressing the mounting base 30 onto the patient's skin. During this step, the insertion needle 14 pierces the patient's skin and carries the cannula 15 with the sensor distal segment 16 therein to the appropriate transcutaneous placement site. During insertion, the sensor distal segment 16 is supportively sandwiched between the needle 14 and a lower interior wall of the cannula 15, with the distal segment 16 projecting at least slightly beyond the window 19 to prevent inadvertent sensor dislocation from within the cannula.

-10-

When the sensor is transcutaneously placed, with the mounting base 30 seated upon the patient's skin, the insertion needle 14 can be withdrawn from the patient. During this withdrawal step, the insertion needle 14 slides from within the cannula 15 and along the sensor distal segment 16, leaving the sensor distal segment 16 with the electrodes 18 thereon at the selected insertion site in alignment with the cannula window 19. The electrodes are thus exposed to patient blood or other body fluid, resulting in signals which are coupled via the conductive contact pads 22 on the sensor proximal segment 20 to the monitor 24. The sensor 12 can thus be used over a prolonged period of time for taking blood chemistry readings, such as blood glucose readings in a diabetic patient.

FIGS. 7-12 show alternative cannula and sensor geometries for exposing sensor electrodes 18 on a sensor distal segment for direct contact with patient body fluid. For convenience, modified structures conforming in function to those previously shown and described are identified in FIGS. 7-12 by primed reference numerals.

More specifically, FIGS. 7 and 8 show a modified cannula 15' at a distal end thereof to include a modified window 19' defined by a pair of spaced-apart radial slits formed in the cannula. In this version, the sensor distal segment 16 (FIG. 11), which can be identical to the sensor shown in FIGS. 1-6, extends within the cannula 15' through the radial slits so that sensor electrodes 18 are located exteriorly of the cannula segment disposed axially between the radial slits. The sensor distal segment 16 projects through both slits, whereby the sensor terminates within the cannula 15' at a location beyond the window 19' to achieve a mechanical

-11-

interlock between the cannula and sensor, to assist in preventing inadvertent sensor dislocation.

FIGS. 9, 10 and 12 show a further modified form wherein a cannula 15" has a window 19" defined by spaced radial slits, but wherein the cannula material between these slits is further cut in the axial direction to define a pair of flaps 62. In this version, the sensor has a modified distal segment 16' with electrodes 18 thereon (FIG. 12), but including an enlarged end tab 64. The sensor distal segment 16' is installed with the electrodes 18 exposed through the window 19", with the flaps 62 located radially inboard thereof, and with the end tab 64 extending beyond the flaps 62 within the cannula 15" to achieve a mechanical interlock which prevents sensor dislocation. In both of the embodiments of FIGS. 7-12, the cannula window is normally oriented downwardly as described previously with respect to FIGS. 1-6, to issue proper alignment with the sensor electrodes 18 when the insertion set is assembled. The insertion needle 14 (not shown in FIGS. 7-12) supports the sensor against the lower interior wall of the cannula during sensor placement on a patient, as previously described.

The transcutaneous sensor insertion set of the present invention thus provides a relatively simple device for quickly and easily placing a flexible thin film electrochemical sensor at a selected position within a patient. The device is assembled quickly and easily, with positive component alignment and orientation being assured.

A variety of modifications and improvements to the transubcutaneous sensor insertion set of the present invention will be apparent to those skilled in the art. As an example, the resilient cannula may be used to deliver a medication to or otherwise withdraw fluid from the patient, in addition to the

-12-

functions of supporting and protecting the sensor. Accordingly, no limitation on the invention is intended by way of the foregoing description and accompanying drawings, except as set forth in the appended claims.

WHAT IS CLAIMED IS:

1. A transcutaneous sensor insertion set, comprising:

a mounting base adapted for mounting onto a patient's skin;

a flexible sensor having a proximal segment carried by said mounting base, and a distal segment protruding from said mounting base and having at least one sensor electrode thereon;

a hollow cannula;

means for supporting said cannula from said mounting base to protrude therefrom with said sensor distal segment received therein and with said cannula in a predetermined orientation relative to said mounting base for positioning at least one window formed in said cannula generally in alignment with said at least one sensor electrode; and

a insertion needle carried by said mounting base to protrude therefrom through said cannula whereby said sensor distal segment is interposed and supported between said needle and an interior wall surface of said cannula, said insertion needle being slidably withdrawable from said mounting base and said cannula to leave said sensor distal segment within said cannula.

2. The transcutaneous sensor insertion set of claim 1 wherein said means for supporting said cannula comprises cooperative mount means on said cannula and said mounting base for orienting said cannula in a predetermined rotational position relative to said mounting base.

-14-

3. The transcutaneous sensor insertion set of claim 2 wherein said cooperative mount means comprises a mounting flange of noncircular cross sectional shape on said cannula and for seated reception into a matingly shaped recess formed in said mounting base.

4. The transcutaneous sensor insertion set of claim 1 further including means for securing said sensor distal segment in position relative to said cannula.

5. The transcutaneous sensor insertion set of claim 4 wherein said securing means comprises forming said sensor distal segment with a length to extend within said cannula to a position axially beyond said window.

6. The transcutaneous sensor insertion set of claim 1 wherein said cannula window is formed by a pair of axially spaced, radially extending slits formed in said cannula, said sensor distal segment extending through said slits to expose a portion of said sensor distal segment to the exterior of said cannula, said exposed portion having said at least one electrode thereon.

7. The transcutaneous sensor insertion set of claim 6 wherein said sensor distal segment includes an enlarged tab at a free end thereof, said tab being positioned within said cannula at a location axially beyond said window.

8. The transcutaneous sensor insertion set of claims 1 wherein said sensor is a flexible thin film sensor.

-15-

9. The transcutaneous sensor insertion set of claim 1 wherein said sensor is an electrochemical sensor.

10. The transcutaneous sensor insertion set of claim 1 wherein said sensor is a glucose sensor.

11. The transcutaneous sensor insertion set of claim 1 wherein said insertion needle extends through an open port formed in said mounting base, said insertion needle being positioned to pierce a patient's skin to carry said cannula with said sensor distal segment therein to an insertion position within the patient upon placement of said mounting base onto the patient's skin, said insertion needle being slidably withdrawable from the patient's skin and said mounting base to leave said cannula with said sensor distal segment therein at the insertion position.

12. The transcutaneous sensor insertion set of claim 1 wherein said mounting base comprises a central housing member for receiving and supporting said cannula and for receiving and supporting said sensor with said proximal segment thereon and with said distal segment disposed angularly relative to said proximal segment to extend into said cannula, a wing member overlying said central housing member and defining oppositely projecting wings for removable attachment to a patient's skin, and a cover plate overlying a portion of said wing member and including attachment means for connection to said central housing member with said portion of said wing member clamped between said cover plate and central housing member.

-16-

13. The transcutaneous sensor insertion set of claim 12 wherein said attachment means comprises snap-fit tabs formed on said cover plate.

14. The transcutaneous sensor insertion set of claim 1 wherein said mounting base further includes an electrical connector element conductively connected to said sensor proximal segment.

15. A transcutaneous sensor insertion set, comprising:

a mounting base adapted for placement onto a patient's skin;

a flexible sensor having a proximal segment carried by said mounting base, a distal segment protruding downwardly from said mounting base and having a tip end with at least one sensor electrode thereon;

a hollow cannula having one end supported by said mounting base and protruding therefrom with said sensor distal segment therein;

cooperative mount means on said cannula and said mounting base for orienting said cannula in a predetermined orientation relative to said mounting base, said cannula having at least one window formed therein for exposing said sensor electrode to patient body fluid; and

an insertion needle slidably receivable through an open port formed in said mounting base to extend through said cannula whereby said sensor distal segment is supported between said needle and an interior wall surface of said cannula;

said insertion needle being positioned to pierce a patient's skin to carry said cannula and said sensor distal segment therein to an insertion position within the patient upon placement of said mounting base onto the patient's skin, said insertion

-17-

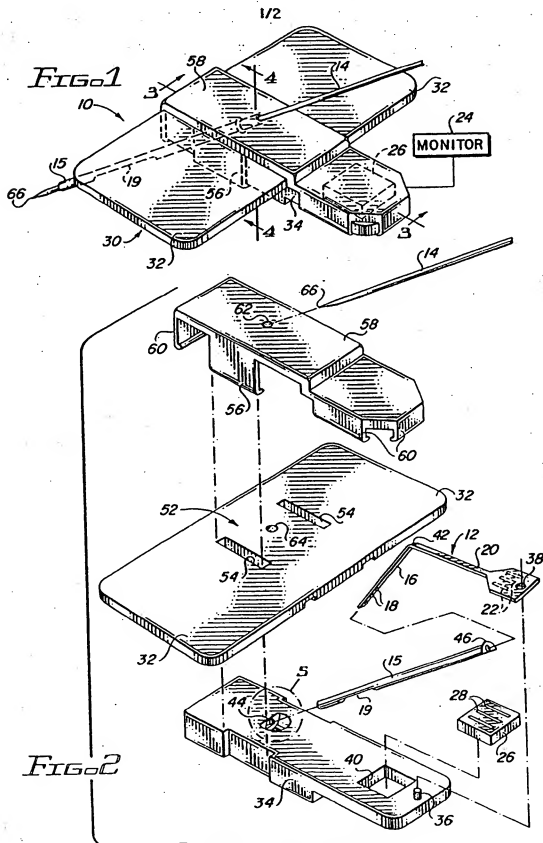
needle being slidably withdrawable from the patient's skin and said mounting base to leave said cannula with said sensor distal segment therein at the insertion position.

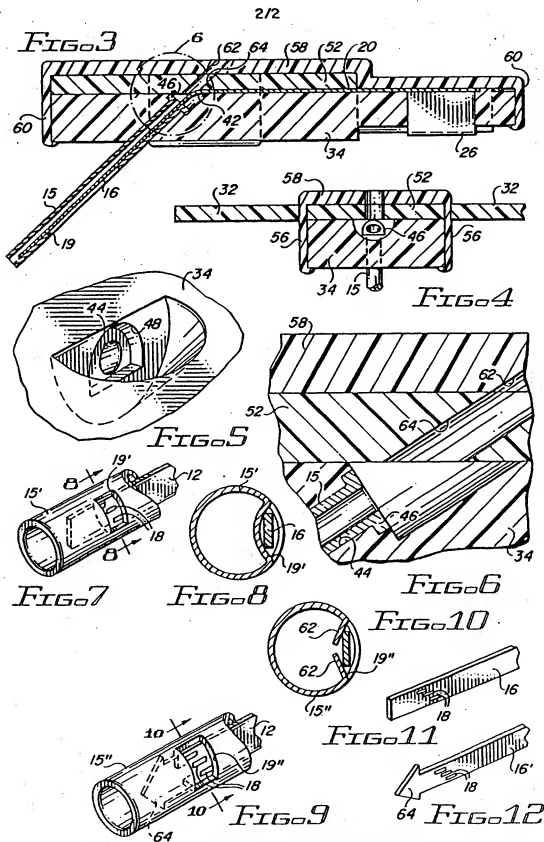
16. The transcutaneous sensor insertion set of claim 15 wherein said mounting base supports said sensor proximal segment in angular relation to said cannula with said sensor distal segment therein.

17. The transcutaneous sensor insertion set of claim 15 further including means for securing said sensor distal segment in position relative to said cannula.

18. The transcutaneous sensor insertion set of claim 15 wherein said mounting base comprises a central housing member for receiving and supporting said cannula and for receiving and supporting said sensor with said proximal segment thereon and with said distal segment disposed angularly relative to said proximal segment to extend into said cannula, a wing member overlying said central housing member and defining oppositely projecting wings for removable attachment to a patient's skin, and a cover plate overlying a portion of said wing member and including attachment means for connection to said central housing member with said portion of said wing member clamped between said cover plate and central housing member.

19. The transcutaneous sensor insertion set of claim 18 wherein said attachment means comprises snap-fit tabs formed on said cover plate.





INTERNATIONAL SEARCH REPORT

International application No.

PCT/US96/02006

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61B 5/00

US CL :128/634

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 128/632, 634, 635, 637, 639-642, 644, 917, 919, Dig 26; 604/49, 51, 52, 158, 160-164, 174, 180

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
NONE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, A, 5,299,571 (MASTROTOTARO) 05 April 1994, see entire document.	1-4, 8-11, 14-17

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be part of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (to specify)	"A" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

08 APRIL 1996

Date of mailing of the international search report

07 MAY 1996

Name and mailing address of the ISA/US

Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

SAMUEL GILBERT

Telephone No. 703-308-3553